MEIOTIC PROPHASE PHENOMENA IN SPECIES AND INTERSPECIFIC HYBRIDS OF NICOTIANA

T. H. Goodspeed

With three plates and one text-figure

INTRODUCTION

Investigations bearing upon problems of species origins and relationships in the genus *Nicotiana* have been carried on in this laboratory at the University of California for many years. With increasing accumulation of evidence it appears that, in this genus at least, extent of chromosome pairing at MI in F_1 interspecific hybrids in general reflects the degree of relationship of the species involved. Recent articles (Goodspeed, 12, 13, 14) expose the extent and character of the data in this and other cytological connections and reveal the close correspondence between morphological, taxonomic, and cytogenetic evidence of relationships within the genus.

Chromosome behavior at MI has been studied in a total of 213 F_1 interspecific hybrids of *Nicotiana*. Information in the case of 135 of these hybrids, which involve as parents 53 of the 58 valid species (Goodspeed, 12, 14; Wheeler, 49), has been obtained in this laboratory, and Kostoff (27) has contributed much of the remainder. Of the 135, 69 are intrasectional, 25 intersectional, and 41 are intersubgeneric hybrids. Our evidence shows that 29 hybrids exhibit at MI complete or almost complete pairing, 35 lack of pairing or approximations thereof, 22 low but variable pairing, 16 high but variable pairing and 35 "Drosera scheme" pairing. Examples of these various categories of pairing are discussed in what follows.

The correlation of extent of MI pairing in hybrids with the taxonomic relationships of the species involved is as follows: in approximately 90% of intrasectional hybrids pairing is complete or nearly so; 90% of intersectional and all intersubgeneric hybrids fall into the lack of pairing category; all hybrids involving amphidiploid species (cf. Goodspeed and Bradley, 16) and the descendants of their putative ancestors show "Drosera scheme" pairing, while 85% of hybrids involving these amphidiploid species and species other than those postulated to have entered into their parentage show almost complete lack of pairing. The information concerning meiotic phenomena in the additional 78 hybrids studied by others enforces almost without exception the significance of the above evidence.

The proposition that amount and character of MI pairing reflects the extent to which in the parental genoms the genes and their arrangement are

the same or similar is obviously basic to all cytotaxonomic conclusions to which studies of first meiotic metaphase chromosome behavior contribute (Stebbins, 44). A considerable to a high degree of MI pairing is characteristic of many interspecific hybrids in most genera other than *Nicotiana* (cf. Stebbins, l. c.). The apparent conclusion that *Nicotiana* is unique in the sense that many interspecific hybrids exhibit a negligible amount of MI pairing is, however, difficult to document. Taxonomic criteria from genus to genus are variable, and thus what is said to constitute an interspecific hybrid in one may correspond to a varietal hybrid in another. Again, in no other genus is comparable cytological evidence available for such a high proportion of the possible interspecific combinations, and there is here the suggestion that more extensive data for other genera might reveal the occurrence of pairing categories comparable to those in *Nicotiana*. This discrepancy in evidence may be, in part at least, a product of inability to obtain interspecific hybrids in other genera, a possibility which suggests that the ability to obtain numerous hybrids between species of *Nicotiana* which are taxonomically remotely related may be due to evolution of factors inhibiting crossibility having occurred at a slower rate than evolution of those responsible for species differentiation. In any case, interspecific hybridization — frequently leading to amphidiploidy with its polyploid and aneuploid byproducts — apparently represents a major evolutionary mechanism in *Nicotiana*, and disappearance of many of the contributory ancestors leaves the modern genus small in terms of species and restricted in terms of distribution. In such a relic genus with such an evolutionary background species distinctions, morphological and thus genic, may be expected to be considerable with the result that lack of pairing and "Drosera scheme" pairing are of relatively frequent occurrence among its F_1 interspecific hybrids. In other words, it is probable that *Nicotiana* may actually be unique among genera which have been cytotaxonomically treated.

Important for the interpretation of the character and significance of MI pairing in F_1 interspecific hybrids is the question of the extent to which pairing observed at MI is a reflection of meiotic prophase association. This is particularly true, for example, in the considerable number of *Nicotiana* hybrids where the pairing mode at MI is zero or approximately zero. It may be contended in such cases that MI evidence is not reliable because genic effects are known to produce desynapsis and thus a complete or considerable zygotene-pachytene association might not necessarily be followed by the appearance of a corresponding amount of MI pairing. Therefore, without evidence concerning early meiotic prophase phenomena in hybrids, the amount of MI pairing is doubtfully applicable to interpretation of the relationships of the parental species involved. In the present article the results of comparative studies of meiotic prophases of species and F_1 interspecific hybrids of *Nicotiana* are described and commented upon.

There are few reports of meiotic prophase phenomena in species or F_1

interspecific hybrids of *Nicotiana*. For triploids of *N. tabacum* Olmo (35) and for haploids of that species, Lammerts (28) described the extent and something of the character of pachytene associations. In a normal haploid plant the average number of bivalents per PMC at MI ranged from .17 to .43, while in a "Coral" *N. tabacum* haploid in which the F chromosome was genetically altered the average was 1.44. At pachytene the correspondingly greater amount of association observed in the "Coral" as contrasted with the normal haploid was assigned primarily to non-homologous pairing. In an asynaptic haploid of *N. sylvestris* (Goodspeed and Avery, 15), approximately 50% of the PMC contained a bivalent at MI with some instances of 2 to 4 bivalents. Some pachytene pairing was seen, primarily between segments of two strands which apparently were structurally alike as a result of duplication. The rare occurrence of more than one bivalent at MI was assigned to non-homologous association and "fold backs" at prophase. In the above citations emphasis at pachytene was laid upon correspondence in chromomere pattern or its absence as indicative of the presence or lack of homology in the paired chromosomes. Some reference to meiotic prophase in haploids of *N. rustica*, *N. Langsdorffii*, and *N. sylvestris* is made by Kostoff (27), who found that the negligible amount of pairing observed at MI was preceded by a minimum amount of pachytene association. Elvers (9) made a preliminary examination of pachytene in F_1 *N. glutinosa* × *N. wigandioides*.[1] At MI this hybrid shows a range of 2 to 9 pairs. At pachytene paired threads appeared to be much more numerous than unpaired ones. In some cases Elvers considered the paired threads homologous in terms of matching chromomeres, while others appeared to be instances of non-homologous association.

Relatively little information is available concerning the relation between prophase and MI chromosome behavior in hybrids in other genera in which pairing at the latter stage of meiosis is lacking or reduced. In most such cases a typical pachytene stage was not seen (cf. Federley, 10; Harrison and Doncaster, 17; Ramanujam, 37) or only short paired segments were observed (Meurman, 32). However, Karpechenko (21) found in hybrids of *Raphanus sativa* × *Brassica oleracea* that synapsis did not differ from that in the parents, although there was no pairing at MI. In an interspecific hybrid in *Crepis* (Tobgy, 48), marked differences in length at MI of the parental chromosomes apparently did not reduce the extent of pachytene association.

The reduced amount of MI pairing in asynaptic and desynaptic plants makes them in that sense comparable to F_1 interspecific hybrids of *Nicotiana* which show lack of or low variable pairing. In desynapsis more or less normal zygotene association is observed or indicated (cf. Koller, 23) to have occurred and is followed by lack of chiasma formation (cf. Beadle,

[1] Not F_1 *N. glutinosa* × *N. tomentosa* as originally reported by Elvers.

2; Catcheside, 5; Levan, 29), while in asynapsis zygotene association is found to be lacking or at a minimum (cf. Huskins and Hearne, 19; Ramaer, 36; Yamomoto, 51). Both phenomena are taken to be genically controlled. The falling apart of chromosomes was seen to occur in late pachytene or between diakinesis and MI (cf. Richardson, 39; Levan, 29; Li, Pao, and Li, 30).

TECHNIQUE

Variations of the conventional paraffin technique did not give adequate pictures of meiotic prophase conditions in PMC of species or F_1 interspecific hybrids of *Nicotiana,* although certain paraffin preparations were useful for comparative purposes. Smears were satisfactory when prepared according to the following techniques:

A. After fixation in 3 parts absolute alcohol to 1 part glacial acetic acid for 20 to 24 hours, the anthers were removed to 70% alcohol, two or three changes of alcohol being made within a period of a few hours. A shorter (12 hour) fixation did not prove to be so satisfactory.

B. Fixation in 1 part chloroform to 1 part of the solution used in fixation A was continued for 24 hours to several days. Anthers transferred to alcohol after a 24 hour fixation showed darker cytoplasm than those left in the fixative for a considerably longer period.

After fixation anthers were smeared in strong iron aceto-carmine. Additional iron from dissecting needles was added to the drop of carmine in which anthers were to be smeared until the stain began to appear purplish. Preparations were alternately heated and pressed until the desired degree of spreading and staining of the chromosomes was achieved. Technique B proved to be superior to technique A for detailed studies of spiralization in loops and segments of chromosomes. The less delicate quality of the staining in technique A, however, made its use more appropriate for investigation of the entire contents of nuclei. Therefore, all drawings except PLATE I, *fig. 6* were made from material prepared according to technique A. Swanson (46) has had success in demonstrating finer structure of early prophase chromosomes in *Tradescantia* after pretreatment with heat. One set of cut inflorescences of several *Nicotiana* species was, therefore, kept in jars of water at approximately 40 C. for 24 hours and another at 32 –35 C. for the same period. In neither case did the results of such pretreatment improve the definition of prophase in our material.

OBSERVATIONS

(a) SPECIES. — Over a period of years information has accumulated in this laboratory concerning diplotene-diakinesis sequences in *Nicotiana* and has been applied to interpretation of chromosome behavior at MI in species and F_1 interspecific hybrids. Until the techniques above described were available, pre-diplotene stages proved difficult to study and little significant evidence on those early meiotic phenomena was secured.

The four species referred to in what follows were selected for description of the leptotene-diakinesis progression because they are distinguished from one another in chromosome number or karyotype or because they represent parents of F_1 hybrids in which meiotic prophases have been studied. Members of Subgenus *Petunioides*, Section *Alatae*, *N. Langsdorffii* (n = 9) has a $2\ m + 4^1\ s\ m + 3^1\ st$ karyotype and *N. longiflora* a $10^2\ st$; *N. glauca* (Subgenus *Rustica*, Section *Paniculatae*) has a $1m + 1\ s\ m + 10^1\ st$ karyotype, while in *N. otophora* (Subgenus *Tabacum*, Section *Tomentosae*) the karyotype is $7^1\ m + 5^1\ st$ (Goodspeed, 13). In a number of instances marked distinctions in somatic chromosome morphology make possible the identification of individual chromosomes in early meiotic prophases and thereby assist in interpretation of the results obtained.

Pre-leptotene chromosomes show relic coils which are eliminated by mid-leptotene along with parallel enlargement of the nucleus and lengthening of individual strands. Adequate evidence of duality and of the relational coiling of sister chromatids resulting from a gradual resolution of relic coiling is not yet available. Optically, therefore, mid-leptotene chromosomes appear as much attenuated, slender chromonemata evenly distributed in the nucleus. With technique A these chromosomes have a distinctly beaded appearance which for certain of them at least seems to correspond to a pattern in terms of size and linear position of chromomeres. With technique B, however, equivalent material shows the establishment at mid-leptotene of the meiotic spiralization cycle. Thus it appears that the beaded appearance of the chromosomes (chromomeres) is largely a product of fixation which obscures the presence of the major spiral without altering spiralization patterns. The "spiralization pattern" of a meiotic prophase chromosome is here assumed to be established by genically controlled distinctions in size and pitch of gyres, in their linear relations, and in amount and/or character of nucleic acids of consecutive segments. In other words, our evidence supports the conclusion that a pattern of chromomeres represents a heritable spiralization pattern. From this point of view the spiralization pattern is the same in sister chromatids and homologous chromosomes. Certainly at zygotene conspicuous linear correspondence of spiralization patterns is often seen, and obviously complete and intimate association between two chromosomes in which the major coils have been developed can occur only where both possess identical spiralization patterns.

At early zygotene conjugating segments are seen at ends of chromosomes and/or in intercalary regions which may represent position of centromeres (Plate I, *fig. 6a, b*). Free ends extending from paired segments frequently can be seen to possess identical spiralization patterns. By late zygotene intermeshing of the two spiralized chromosomes is complete and only at ends (particularly at satellites) can the dual nature of the strands be demonstrated. Some relational coiling occurs at pachytene and appears to represent random twisting of the long paired threads. During pachytene appreciable condensation is seen. This is a product of the onset of

despiralization which begins to be conspicuous at late pachytene, where in chromosomes undergoing repulsion the number of gyres is reduced, the diameter of the gyres is increased, and the gyres are closer together (PLATE I, *fig. 6 c, d*).

At mid-pachytene distinctions in somatic chromosome morphology within the genoms of the species of *Nicotiana* under discussion permit identification of entire paired lengths or large segments of such lengths. Thus, in a number of PMC of *N. Langsdorffii* four of the nine pairs can be individually distinguished (cf. PLATE I, *fig. 2*). For example, the entire extent of one submedian pair characterized by possession of an extremely large satellite, and of one very short subterminal pair with distal satellites, could be studied and were found consistently associated with the nucleolus. In this species at pachytene centromeric as well as satellite constrictions are pronounced and usually reflect the duality obtaining. In *N. longiflora* (PLATE I, *fig. 3*) the two chromosomes which bear satellites, one a small proximal and the other a large distal satellite, and the nucleolus were always associated. Even at this stage these two chromosomes were distinguishable from each other by reason of size and position of satellites. Another somatic chromosome of this species characterized by a large distal knob is readily identifiable at pachytene by the presence of a conspicuous terminal heterochromatic area. Although during mitosis the knob never appears as a typical satellite it is at pachytene frequently, though not consistently, found near the nucleolus. In *N. longiflora* centromeric constrictions at pachytene, unlike those of *N. Langsdorffii,* are not pronounced in the majority of the chromosomes. As in *N. Langsdorffii* and *N. longiflora,* so also in *N. glauca* (PLATE I, *fig. 1*) and *N. otophora* certain chromosomes at pachytene can be identified by distinctions in centromere position and or by number and size of satellites, and in the latter species certain chromosomes of both length classes of the strikingly dimorphic genom can be followed over their entire extent.

In early diplotene condensation has reached the point where, in such species of low chromosome number as *N. Langsdorffii* and *N. longiflora* (PLATE I, *fig. 4*), the majority of the paired lengths can be studied in their entirety. However, relational coiling due to twisting of homologues makes impossible a determination of all points at which crossing-over has occurred. As diplotene advances, further despiralization, accompanied by maximum attraction and repulsion of homologues and chromatids, is apparent with consequent decrease in relational coiling. As a result chiasmata in the now optically quadripartite units become readily distinguishable from twists which did not involve crossing-over (PLATE I, *fig. 4*). Often chromatids can be traced through chiasmata and the independent coils of sister chromatids identified (PLATE I, *fig. 6 g*). At diakinesis (PLATE I, *fig. 5*) the tetrads, distributed about the periphery of the nucleus, become exceedingly compact and are characterized by outlines the distinctly uneven

quality of which indicates the presence of spiralization which is obscured by accumulation of matrical material.

(b) Hybrids. — In all F_1 interspecific hybrids of *Nicotiana* except those characterized by approximately complete MI pairing the meiotic prophase sequence, particularly mid to later stages, shows marked departures from that of the parental species, the extent of the distinctions being in general directly proportional to the extent to which the chromosomes fail to pair at MI.

As in other genera, the leptotene-diakinesis sequence in species of *Nicotiana* exhibits stages which become points of reference. Thus, zygotene with its tendency toward parallel orientation of homologues and its evidence of the beginning of synaptic unions, mid-pachytene where all chromosomes exhibit an approximately uniform degree of condensation and are completely paired with duality apparent only at ends or where repulsion is already under way, and diplotene with its conspicuous configurations can all be identified with confidence. By contrast, in F_1 interspecific hybrids such points of reference may be much less pronounced or entirely lacking. For example, in hybrids of the lowest pairing category there is nothing comparable to zygotene, pachytene is identifiable only in PMC where certain chromosomes show intimate association over relatively short segments, and typical diplotene does not occur.

In hybrids, as in species, pre-leptotene chromosomes exhibit relic coiling. As leptotene strands take form, this coiling is wholly or largely lost and by mid-leptotene appears to be replaced by the initiation of the new major coiling cycle. As in species, the leptotene material of hybrids prepared according to technique A showed bead-like chromomeres which after technique B was applied to equivalent material were seen as gyres of a specific spiralization pattern. Although not yet adequately demonstrated, it appears that in hybrids, particularly of the low pairing category, there is a somewhat stronger suggestion than in species of duality of early to mid-leptotene chromosomes. Assuming leptotene duality, relational coiling of sister chromatids resulting from straightening of relic coiling must have been eliminated, probably, in part, by rotation of ends in the enlarging nucleus and in part by the initiation independently in the closely appressed sister chromatids of the major coiling cycle. In general, except for absence of typical zygotene, pre-pachytene conditions in hybrids correspond to those in species.

At pachytene, however, striking contrasts appear. Whether pachytene pairing in a given nucleus of a hybrid is approximately complete or is limited to a few short segments or is entirely lacking, the unpaired chromosomes (whole chromosomes or segments) are strikingly atypical as a result of the occurrence of alternating thick and thin areas of varying length. This phenomenon continues until diakinesis which, apart from differences in the valencies of the chromosomes involved, is entirely comparable in appearance to the same stage in species. The presence of spiralization

patterns in the chromosomes throughout the meiotic prophase is more readily demonstrated in hybrids than in species, at least in the sense that it is revealed after technique A as well as after B.

As a typical representative of a *Nicotiana* hybrid exhibiting lack of pairing at MI, the meiotic prophases of F_1 *N. glauca* ($n = 12$) × *N. plumbaginifolia* ($n = 10$) were extensively studied (PLATES II, III). Apart from the report of Kostoff (27), more than 4 pairs at MI have not been seen in this hybrid. Some 750 PMC analyzed by Ramanujam and Joshi(38) gave a pairing range of 0 to 4, well over 75% showing zero pairs, and our unpublished data involving over 100 PMC correspond (*Text fig. 1, a*). On the other hand, Kostoff (l. c., p. 396) reports a pairing range of 3 to 9 and elsewhere (l. c., p. 632) one of 6 to 10. It should be noted that the occurrence of numerous "off-spindle attachments" and the tendency of the univalents to form an equatorial plate rather than lying scattered in the PMC produces a misleading impression of the amount of pairing obtaining.

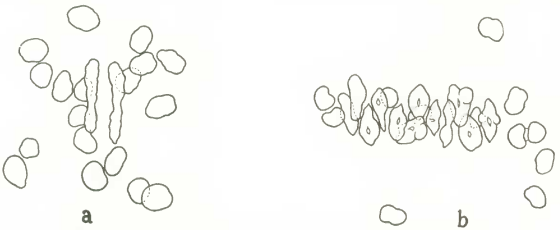


FIG. 1. MI conditions in F_1 interspecific hybrids: *a*, F_1 *Nicotiana glauca* (n = 12) × *N. plumbaginifolia* (n = 10), showing 2 bivalents and 18 univalents, off-spindle attachments, and secondary association; *b*, F_1 *N. tabacum* (n = 24) × *N. otophora* (n = 12), showing 12 bivalents and 12 univalents.

In this hybrid some PMC at a stage taken to correspond to pachytene showed only unpaired chromosomes, but in the majority of PMC from one to several paired segments, frequently but not exclusively terminal, occurred (PLATE II, *figs. 2, 3*). Usually such paired segments were short, but in most favorable material a relatively long paired segment was sometimes seen.[2] In terms of identity of spiralization patterns of the segments paired at pachytene and of distinctions of such patterns in unpaired segments of the same chromosomes, conjugation appears to occur only between structurally homologous segments of otherwise non-homologous chromosomes (PLATE II, *fig. 3*). This conclusion is borne out by the occasional presence of heteromorphic pairs at MI in this hybrid.

[2] Undoubtedly this pairing is of sufficient length to permit chiasma formation, a fact which probably accounts for the relatively frequent occurrence of one pair at MI.

At diplotene (cf. PLATE II, *figs. 4, 5, 6*) the duality of each chromosome is rather strikingly visible, particularly in terminal areas (PLATE II, *fig. 5*), sister strands showing as independently coiled elements capable of lateral separation from each other. At early diakinesis (PLATE III, *fig. 1*) evidence of duality and spiralization is unmistakable, whereas in species at an equivalent stage both are somewhat difficult to demonstrate. In the hybrid centromeric constrictions are conspicuous. Frequently the initiation of off-spindle attachments and of secondary association characteristic of some univalents at MI can be seen (PLATE III, *fig. 2*), reflecting perhaps an earlier association of segments too short to permit chiasma formation.

In the case of F_1 *N. tabacum* ($n = 24$) $\times$ *N. otophora* ($n = 12$), a hybrid which combines the genoms of the former species and of a modern descendant of one of its putative ancestors, MI shows a close approximation of the "Drosera scheme" pairing which is characteristic of other hybrids which, like it, involve amphidiploid species and those related to their parentage. The pairing mode in some 150 PMC analyzed is $12_{II} + 12_I$ although a range of 10 to 13 pairs occurs, with a trivalent frequently seen (*text fig.* 1, b). As in F_1 *N. glauca* $\times$ *N. plumbaginifolia,* a lack of pairing hybrid, detailed prophase studies of this "Drosera scheme" one reveal a complete correspondence between the amount of pachytene and MI conjugation (PLATE III, *figs. 3, 4, 5*). Thus at the former stage paired and unpaired chromosomes appear in approximately a 1:1 ratio (PLATE III, *fig. 3*), although it cannot be determined as accurately as can the MI ratio of bivalents to univalents because of the difficulty of following any one of the pachytene chromosomes throughout its entire length. However, complete pairing is clearly visible over the entire extent of large pachytene loops which, in some instances at least, represent the major portions of the chromosomes involved, and the occurrence of long unpaired lengths is equally conspicuous. Unpaired segments in otherwise completely paired strands are seen at times. In them the spiralization patterns are not the same, whereas paired chromosomes consistently appear to be structurally homologous. Some relational coiling of homologues was observed.

As will be noted in PLATE III, *fig. 3,* satellited chromosomes — two paired and one unpaired — are attached to the nucleolus, and there are not too sharply defined heterochromatic regions, the latter doubtless contributing the chromocenters which are peculiarly conspicuous in somatic nuclei of *N. otophora* at the metabolic stage.

Pairing at MI in F_1 *N. paniculata* ($n = 12$) $\times$ *N. Benavidesii* ($n = 12$) is approximately as complete as it is in the parental species with a mode of 12_{II} in 85 of the 100 PMC analyzed. At pachytene, in the many cells examined, no unpaired chromosomes or segments were found. Another hybrid of the complete pairing category, F_1 *N. Raimondii* ($n = 12$) $\times$ *N. cordifolia* ($n = 12$), is important as indicating the degree of reflection of MI conjugation at pachytene, since at MI the pairing mode is $11_{II} + 2_I$ and in pachytene, although completely paired lengths are the

rule, unpaired segments can at times be seen. Throughout the meiotic prophase sequence of the complete pairing hybrids all stages appeared to correspond in detail to equivalent stages in the parental species.

For the hybrid F_1 *N. tabacum* ($n = 24$) $\times$ *N. glauca* ($n = 12$) the extent of MI pairing has been variously reported. Sarana (41) mentions "up to 12 pairs," Kostoff (27) gives a pairing range of 9 to 12, while in approximately 100 PMC analyzed here the range was 0 to 8 with 4 and 5 pairs occurring with equal frequency. This hybrid has therefore been placed in the low variable pairing category and prophase investigation makes it clear that association at pachytene is similarly low but variable in amount. Although unpaired chromosomes predominate in the majority of the PMC there is considerable variation from one cell to the next, but no instance of "Drosera scheme" or even approximately "Drosera scheme" pairing occurs.

Prophase conditions in an asynaptic individual of *N. tomentosa* ($n = 12$) were studied, since under the influence of environmental conditions variability in extent of MI pairing was comparable to that of such a hybrid as has just been described. When pachytene and MI material were taken simultaneously from this plant the latter stage was a reflection of the former in terms of the amount of pairing which occurred. It should be noted, however, that even when MI pairing was minimum the majority of PMC at pachytene showed, by contrast with F_1 interspecific hybrids of *Nicotiana* of the lack of pairing category, a considerable number of paired segments and even what were taken to be one or more completely paired chromosomes (cf. PLATE III, *fig. 6*).

COMMENT AND SUMMARY

Comparative studies of meitoic prophase phenomena in species and F_1 interspecific hybrids of *Nicotiana* above described show that in both cases the extent and quality of MI pairing is a reflection of the amount of early prophase association. Thus, in a hybrid showing approximately as complete pairing at MI as occurs in the parental species, zygo-pachytene conjugation is also complete. In a hybrid exhibiting a variable amount of pairing from one PMC to the next at MI, an equivalent range in ratio of paired to unpaired chromosomes appears throughout the zygotene-diakinesis sequence. Similarly, where "Drosera scheme" behavior is shown at MI in a hybrid where the chromosome number of one parent is twice that of the other, the ratio at pachytene between paired and unpaired units is approximately 1:1. The extensive studies of prophase phenomena in hybrids showing complete or almost complete lack of pairing in all PMC analyzed at MI confirm the evidence just summarized that univalents at MI reflect absence of prophase association or that such association is commonly confined to short segments.

There is no evidence that genically conditioned desynapsis is responsible

for the univalents present at MI in F_1 interspecific hybrids[3] nor that their occurrence can be assigned to the presence of an inherited asynaptic state. If a genic alteration causing asynapsis is recessive, its effects would not be manifest in the hybrids under discussion. It is, of course, possible that dominant gene mutations leading to asynapsis might offer an explanation for a few of the many instances of complete lack of pairing or variable pairing at MI, but "Drosera scheme" behavior obviously could not be assigned to the operation of such heritable influences. Furthermore, the fact that distinctions in relationship based upon morphology and distribution are almost uniformly in accord with cytogenetic evidence is significant in this entire connection.

Chromomeres have been variously described and interpreted. For example, they have been referred to as discrete chromatic disks of varying thickness (Heilborn, 18), as a series of enlargements of the genonema (Koltzoff, 25), condensation centers of chromatin (Ellenhorn, 8), localization centers of nucleic acids (Caspersson, 4), products of close intertwining of sister chromatids (cf. Kaufmann, 22), and as misinterpretations of coiled structures (Ris and Crouse, 40). In *Nicotiana* interpretation of chromomeres and their disposition as evidence of the presence of specific spiralization patterns appears justified. As already indicated a spiralization pattern is here taken to represent the product of genically controlled distinction in size and pitch of gyres, in their linear relations, and in the quantity and character of nucleic acids in consecutive segments.

In the species of *Nicotiana* examined leptotene duality is not demonstrable. However, at lepto-zygotene each chromosome is here assumed to represent a double strand the sister chromatids of which have been freed from relational coiling by rotation as they earlier straightened and lengthened and by initiation of the major coil independently in each chromatid. Onset of spiralization at leptotene is suggested by the evidence of Taylor (47), Shinke (43), Koshy (26), Naithani (34), and Swanson (46), whereas Darlington (7) and Huskins and Smith (20) see leptotene as unspiralized.

Following zygotene intermeshing of chromosomes, the homology of which determines an identity of their spiralization patterns, the pachytene-diakinesis sequence becomes a product of despiralization, the operation of forces of repulsion and attraction and the addition of nucleic acids, each of these phenomena characterized by its specific timing relation to the complete progression. Despiralization beginning in pachytene is visible at late pachytene in the greater diameter, as compared with zygotene, of the separating chromosomes and continues through diplotene (PLATE I, *fig. 6e, f*) and diakinesis to effect a progressive decrease in number of gyres and increase in their diameter. This evidence for *Nicotiana* agrees with Swanson's (46) conclusion for *Tradescantia* but is in contrast to that of

[3] Such "desynapsis" as occurs corresponds only to the falling apart of short segments associated at pachytene in which the homology does not extend over a sufficient distance to favor chiasma formation.

certain investigators who see indication of comparable despiralization only at a considerably later stage. The assumption that despiralization is beginning independently in each chromatid of the tetrad during pachytene, concurrently with the occurrence of crossing-over and chiasma formation between chromatids of homologues, suggests that the breaks which condition the latter phenomenon may be in part a result of the tensions set up by the former process. This same assumption provides an explanation of the observed reduction in relational coiling of homologues, decrease in chiasma frequency, and increase in terminalization coefficient characteristic of diakinesis as contrasted with diplotene (Swanson, 45). It appears that the degree of despiralization observed is sufficient to account for the amount of reduction in length of strands which is seen between pachytene and diakinesis without employing axial contraction of the chromosomes as a contributing factor. In *Nicotiana* there is during prophase no evidence of the minor coil which is referred to elsewhere and considered (cf. Sax, 42) as a third factor in effecting reduction in chromosome length.

To analysis of the factors involved in the progress from leptotene to diakinesis, the study of prophases of hybrids, particularly those of the lack of pairing category, makes contributions. As shown above such material where the parental chromosome numbers are low is peculiarly valuable for interpretation of spiralization phenomena. The unpaired chromosomes of both the hybrids and the asynaptic under discussion consistently exhibit striking distinctions in the width of alternating segments (of varying length), a condition undoubtedly proceeding from a disruption of the timed progression of the spiralization-despiralization cycle characteristic of normal species. Investigations of similar material in other genera reveal prophase irregularities. For example, in asynaptics Huskins and Hearne (19) refer to a "confused irregularly contracted zygotene-diakinesis condition," Beadle (1) refers to "local regions of greater condensation," and Ramaer (36) to "a mass of threads partly contracted . . . . " Similarly for F_1 hybrid prophases reference is made to "many fine threads with thickenings at some places . . . [perhaps] the result of differential contraction of threads" (Ramanujam, 37), "general thickening of threads with irregularly alternating regions becoming attenuated and strained in appearance" (Melburn, 31), to the fact that in some loops very thin regions connect thick ones (Cretschmar, 6), and that "the chromosomes undergo successive changes at different rates" (Meurman, 32). Lack of uniform timing of despiralization appears, in large part, to account for the presence in *Nicotiana* hybrids and in an asynaptic of alternating thick and thin segments of varying length in the unpaired chromonemata. Thus, precocious despiralization produces increased width in one segment while segments of the same chromosome retain their relatively attenuated pre-pachytene appearance. In PLATE II, *fig. 4* the presence of a coil with gyres varying in diameter and degree of relaxation can be seen.

There is probably a relation between the timing of the spiralization-

despiralization cycle and the progression of nucleic acid condensation during prophase. For example Callan (3) concludes that nucleic acid is always present during spiralization, White (50) suggests that since chromatids are regarded as more tightly coiled at metaphase when the nucleic acid concentration is taken to be at a maximum, spiralization may be a consequence of nucleic acid synthesis, and Goldschmidt and Kodani (11) refer to coiling or molecular folding of the chromonemata forming the core of the disks of salivary gland chromosomes and to the presence of nucleic acid in the disks and its probable absence between them. It is, therefore, possible that disruption of normal spiralization-despiralization in hybrids might fundamentally represent a disruption of the normal nucleic acid cycle. In this connection it is to be noted that the nucleolus may be concerned in nucleic acid metabolism (cf. Koller, 24), and presumably the balance between the rôles played by nucleoli, chromosomes, and plasma is a specific character. If this is the case, the presence of nucleoli of unrelated origins, and thus presumably of distinctions in amount and activity of nucleolar material, in the nucleus of an interspecific hybrid might directly influence the nucleic acid cycle. Indeed, the physiological condition of the hybrid protoplast as a whole, which is the product of interaction of often large distinctions in the genic constitution of the parental genoms, might affect nucleic acid synthesis. Since in species of *Nicotiana* at pachytene, areas known to be heterochromatic because of connection with centromeres show only a slight differential reaction to staining, the distribution of heterochromatin may not be limited to centromeric regions and satellites (cf. Morgan, Schultz, and Curry, 33). Thus, the thicker and denser portions of the chromosomes above referred to as characteristic of prophases of lack of pairing hybrids might be related to distinctions or transitions between eu- and heterochromatin or, at least, the presence of such chromatic distinctions might intensify the appearance of disruption of the normal pachytene-diakinesis sequence.

The above interpretation of prophase phenomena in F_1 interspecific hybrids of *Nicotiana* has introduced a series of problems which require for solution more evidence than is at present available. Irrespective of the significance which may attach to these phenomena in terms of spiralization-despiralization, nucleic acid and heterochromatin cycles or states, the importance of the results of the comparative prophase studies above described lies in their application to the relation between MI pairing and prophase association. As already stated, the significance of MI association in terms of its indication of fundamental relationships between the parents of F_1 interspecific hybrids cannot be fully accepted without evidence from preceding prophases that such pairing or its lack reflects synapsis or its absence. The investigations discussed above supply such evidence. Therefore, on the basic assumptions concerning the factors responsible for synapsis, it appears that the amount of MI pairing in interspecific hybrids, of *Nicotiana* at least, may be taken as a measure

of the degree to which the genes of the parental genoms united in those hybrids are equivalent or similar in character and arrangement. In other words, in *Nicotiana* the extent and quality of MI association represents a reliable cyto-taxonomic criterion suggestive of phylogenetic relationships.

ACKNOWLEDGEMENTS

The investigations reported upon here were aided by grants from the American Philosophical Society and the Committee on Research, University of California, Berkeley. The assistance of Dr. Muriel V. Bradley and Mildred C. Thompson during these investigations and in the preparation of the manuscript is gratefully acknowledged.

LITERATURE CITED

1. Beadle, G. W. Genetical and cytological studies of Mendelian asynapsis in *Zea mays*. N. Y. (Cornell) Agr. Expt. Sta. Mem. 129, 23 pp. 1930.
2. ———. Further studies in asynaptic maize. Cytologia 4: 269–287. 1933.
3. Callan, H. G. Heterochromatin in *Triton*. Proc. Roy. Soc. London, B. 130: 324–335. 1942.
4. Caspersson, T. Über die Rolle der Desoxyribosenukleinsaure bei der Zellteilung. Chromosoma 1: 147–156. 1939.
5. Catcheside, D. G. An asynaptic *Oenothera*. New Phytol. 38: 323–334. 1939.
6. Cretschmar, M. Das Verhalten der Chromosome bei der Spermatogenese von *Orgyia thyellina* Btl. und *antiqua* L., sowie eines ihrer Bastarde. Zeit. f. Zellf. u. mikr. Anat. 7: 290–399. 1928.
7. Darlington, C. D. The internal mechanics of the chromosomes. I. The nuclear cycle in *Fritillaria*. Proc. Roy. Soc. London, B. 118: 33–59. 1935.
8. Ellenhorn, J. The chromomeres as an indicator of the morphological properties of mitotic chromosomes. Biol. Zhurn. 6: 642–644. 1937.
9. Elvers, I. Interspecific hybridization in *Nicotiana*. XIV. The cytology of F_1 *glutinosa* × *tomentosa*. Univ. Calif. Publ. Bot. 17: 341–354. 1934.
10. Federley, H. Das Verhalten der Chromosomen bei der Spermatogenese der Schmetterlinge *Pygaera anachoreta*, *curtula* und *pigra* sowie einiger ihrer Bastarde. Zeit. f. induk. Abstamm. 9: 1–110. 1913.
11. Goldschmidt, R., and M. Kodani. The structure of the salivary gland chromosomes and its meaning. Am. Nat. 76: 529–551. 1942.
12. Goodspeed, T. H. Studies in *Nicotiana*. III. A taxonomic organization of the genus. Univ. Calif. Publ. Bot. 18: 335–344. 1945.
13. ———. Chromosome number and morphology in *Nicotiana*. VII. Karyotypes of fifty-five species in relation to a taxonomic revision of the genus. Univ. Calif. Publ. Bot. 18: 345–370. 1945.
14. ———. Cytotaxonomy of *Nicotiana*. Bot. Rev. 11: 533–592. 1945.
15. ——— and P. Avery. Trisomic and other types in *Nicotiana sylvestris*. Jour. Genet. 38: 381–458. 1939.
16. ——— and M. V. Bradley. Amphidiploidy. Bot. Rev. 8: 271–316. 1942.
17. Harrison, J. W. H., and L. Doncaster. On hybrids between moths of the Geometrid subfamily Bistoninae, with an account of the behaviour of the chromosomes in gametogenesis in *Lycia* (*Biston*) *hirtaria*, *Ithysia* (*Nyssia*) *zonaria* and in their hybrids. Jour. Genet. 3: 229–248. 1914.

18. Heilborn, O. Further contributions to a chromomere analysis of *Lilium*. Hered. 26: 100–106. 1940.

19. Huskins, C. L., and E. M. Hearne. Meiosis in asynaptic dwarf oats and wheat. Jour. R. Micr. Soc. 53: 109–117. 1933.

20. ——— and S. G. Smith. Meiotic chromosome structure in *Trillium erectum*. Ann. Bot. 49: 119–150. 1935.

21. Karpechenko, G. D. Hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. Jour. Genet. 14: 375–396. 1924.

22. Kaufmann, B. P. Chromosome structure in relation to the chromosome cycle. Bot. Rev. 2: 529–553. 1936.

23. Koller, P. C. Asynapsis in *Pisum sativum*. Jour. Genet. 36: 275–306. 1938.

24. ———. Origin of malignant tumour cells. Nature 151: 244–246. 1943.

25. Koltzoff, N. K. The structure of the chromosomes and their participation in cell metabolism. Biol. Zhurn. 7: 44–46. 1938.

26. Koshy, T. K. Chromosome studies in *Allium*. II. The meiotic chromosomes. Jour. Roy. Micr. Soc. 54: 104–120. 1934.

27. Kostoff, D. Cytogenetics of the genus *Nicotiana*. Karyosystematics, genetics, cytology, cytogenetics, and phylesis of tabaccos. Sofia. 1941–1943.

28. Lammerts, W. E. On the nature of chromosome association in *N. tabacum* haploids. Cytologia 6: 38–50. 1934.

29. Levan, A. The cytology of *Allium amplectens* and the occurrence in nature of its asynapsis. Hered. 26: 353–394. 1940.

30. Li, H. W., W. K. Pao, and C. H. Li. Desynapsis in the common wheat. Am. Jour. Bot. 32: 92–101. 1945.

31. Melburn, M. C. Heterotypic prophases in the absence of chromosome pairing. Canadian Jour. Res. 1: 512–527. 1929.

32. Meurman, O. Cytological studies in the genus *Ribes* L. Hered. 11: 289–356. 1928.

33. Morgan, T. H., J. Schultz, and V. Curry. Investigations on the constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Year Book No. 40: 282–287. 1941.

34. Naithani, S. P. Chromosome studies in *Hyacinthus orientalis* L. II. Meiotic chromosomes. Ann. Bot. n. s. 1: 257–276. 1937.

35. Olmo, H. P. Prophase association in triploid *Nicotiana tabacum*. Cytologia 5: 417–431. 1934.

36. Ramaer, H. Cytology of *Hevea*. Thesis. University of Utrecht. 1935.

37. Ramanujam, S. Cytogenetical studies in the Oryzeae. III. Cytogenetical behavior of an interspecific hybrid in *Oryza*. Jour. Genet. 35: 223–258. 1937.

38. ——— and A. B. Joshi. Interspecific hybridization in *Nicotiana*. A cytogenetical study of the hybrid *N. glauca* Grah. × *N. plumbaginifolia* Viv. Indian Jour. Gen. and Pl. Breed. 2: 80–97. 1942.

39. Richardson, M. M. Meiosis in *Crepis*. II. Failure of pairing in *Crepis capillaris* (L.) Wallr. Jour. Genet. 31: 119–143. 1935.

40. Ris, H., and H. Crouse. Structure of the salivary gland chromosomes of diptera. Proc. Nat. Acad. Sci. 31: 321–327. 1945.

41. Sarana, M. O. Polygenom hybrid *N. glauca* × *N. Tabacum* and *N. Tabacum* × *N. glauca*. Krasnodar. Vses. n.-i. Inst. Tab. i Makh. Prom. Trudy No. 139: 145–197. 1939.

42. Sax, K. Chromosome structure in the meiotic chromosomes of *Rhoeo discolor* Hance. Jour. Arnold Arb. 16: 216–224. 1935.

43. SHINKE, N. Spiral structure in meiosis in *Sagittaria Aginashi*. Mem. Coll. Sci., Kyoto Imp. Univ. B. 9: 367–392. 1934.

44. STEBBINS, G. L., JR. The cytological analysis of species hybrids. II. Bot. Rev. 11: 463–486. 1945.

45. SWANSON, C. P. Some considerations on the phenomenon of chiasma terminalization. Am. Nat. 76: 593–610. 1942.

46. ———. The behavior of meiotic prophase chromosomes as revealed through the use of high temperatures. Am. Jour. Bot. 30: 422–428. 1943.

47. TAYLOR, W. R. Chromosome studies on *Gasteria*. III. Chromosome structure during microsporogenesis and postmeiotic mitosis. Am. Jour. Bot. 18: 367–386. 1931.

48. TOBGY, H. A. A cytological study of *Crepis fuliginosa, C. neglecta*, and their F_1 hybrid, and its bearing on the mechanism of phylogenetic reduction in chromosome number. Jour. Genet. 45: 67–111. 1943.

49. WHEELER, H. M. A contribution to the cytology of the Australian–South Pacific species of *Nicotiana*. Proc. Nat. Acad. Sci. 31: 177–185. 1945.

50. WHITE, M. J. D. The heteropycnosis of sex chromosomes and its interpretation in terms of spiral structure. Jour. Genet. 40: 67–82. 1940.

51. YAMAMOTO, Y. Reifungsteilungen bei einer asynaptischen Pflanze von *Rumex acetosa* L. Bot. and Zool. 2: 1160–1168. 1934.

EXPLANATION OF PLATES

All figures drawn with camera-lucida and reduced to × 1360.

PLATE I

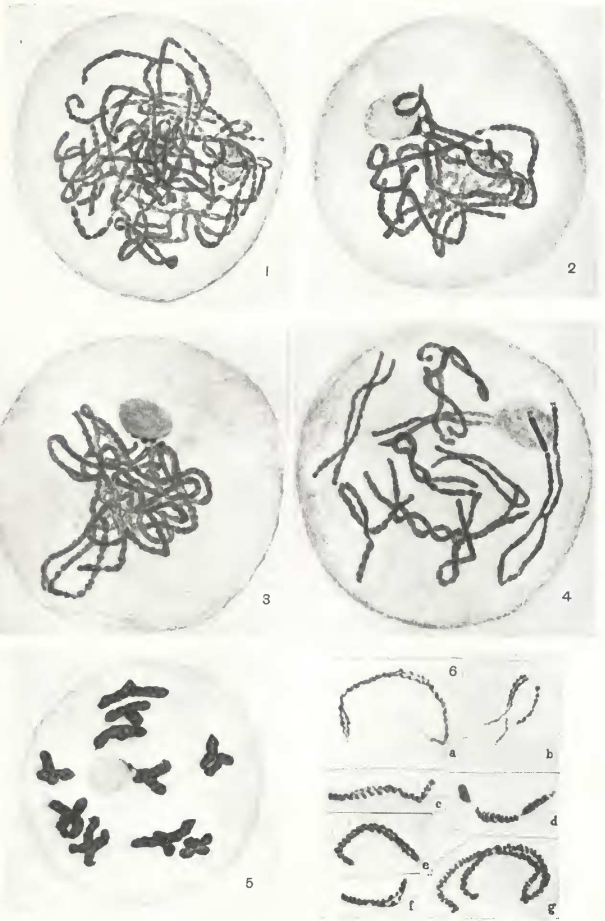
Meiotic prophases of species of *Nicotiana*.

FIG. 1. Pachytene, *Nicotiana glauca* (n = 12): paired chromosomes exhibiting duality at ends and centromeres. FIG. 2. Pachytene, *N. Langsdorffii* (n = 9): four pairs distinguishable in their entirety — two satellited, one long *m* (above), and one *st* (near nucleolus). FIG. 3. Pachytene, *N. longiflora* (n = 10): two satellited pairs associated with nucleolus, another with distal knobs near nucleolus. FIG. 4. Diplotene, *N. longiflora*: all pairs distinguishable throughout their lengths; structure of some chiasmata visible. FIG. 5. Diakinesis, *N. longiflora*. FIG. 6. Zygotene to late diplotene (segments), *N. Langsdorffii*. (*a*), (*b*) zygotene: (*a*) pairing practically complete; (*b*) pairing in centromere area. (*c*), (*d*) pachytene showing onset of despiralization: (*c*) in homologues; (*d*) in segment (including centromere) of pair with large satellite. (*e*), (*f*) early diplotene: identity of spiralization patterns (*f*) in repulsed segment. (*g*) later diplotene: structure of chiasmata and character of spiralization in an entire chromosome pair.

PLATE II

Meiotic prophase of F_1 *Nicotiana glauca* (n = 12) × *N. plumbaginifolia* (n = 10).

FIG. 1. Pre-pachytene. FIG. 2. Pachytene: several short segments associated, unpaired chromosomes exhibiting thick and thin areas. FIG. 3. Later pachytene: distinctions between thick and thin areas more striking; note spiralization in paired segment on extreme right. FIG. 4. Late "pachytene" or early "diplotene": thick and thin areas and spiralization conspicuous. FIG. 5. "Diplotene": chromatid separation apparent. FIG. 6. Diplotene: one paired segment (lower right) possibly with chiasma.



Meiotic Prophase Phenomena in Nicotiana